Mechanism of Late In-Stent Restenosis After Implantation of a Paclitaxel Derivate–Eluting Polymer Stent System in Humans

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Background—We recently reported delayed angiographic restenosis in 15 patients who received 7-hexanoyltaxol (QP2)–eluting polymer stents (QuaDS) for the treatment of in-stent restenosis. This study presents the histological findings of atherectomy specimens from a subset of these patients receiving implants.

Methods and Results—Between October and December 2001, 5 patients treated with QuaDS-QP2 stents underwent directional coronary atherectomy at 11.2±1.0 months for recurrent in-stent restenosis. Restenotic lesion composition was assessed with special stains, immunohistochemistry with quantitative image analysis, and, in one specimen, transmission electron microscopy. Atherectomy specimens contained fibrin interspersed in a smooth muscle cell–rich neointima with proteoglycan matrix. In 2 of 5 specimens, large aggregates of macrophages and T-lymphocytes were noted. These areas of active inflammation demonstrated a relatively high proliferation index by Ki-67 antibody staining, whereas the proliferation index in smooth muscle cell–rich restenotic areas was low.

Conclusion—Restenotic lesions from QuaDS-QP2–eluting stents at 12 months show persistent fibrin deposition with varying degrees of inflammation. These pathological changes, representing delayed healing, are usually observed up to only 3 months in human coronary arteries with stainless steel balloon-expandable stents. The nonreabsorbable polymer alone may have induced chronic inflammation. (Circulation. 2002;106:2649-2651.)

Key Words: fibrin ▪ inflammation ▪ stents ▪ restenosis

Clinical trials with sirolimus-1,2 and paclitaxel-eluting stents (EvaLuation of pacliTaxel-Eluting Stent and Double-blind Comparison of NIR Stent Coated with Paclitaxel in a Polymer Carrier in De Novo Coronary Lesions Compared with Uncoated Controls, unpublished data, 2002) have been reported to virtually eliminate in-stent restenosis in patients with de novo coronary ath erosclerosis at 6 to 12 months. Although similar efficacy has been demonstrated in animals at 28 days, long-term studies with sirolimus have been negative, whereas those with paclitaxel have been inconsistent. The absence of a sustained effect in animals is attributed to delayed healing as observed by persistent intimal fibrin deposition and/or inflammation and incomplete endothelialization at 28 days.3 We recently reported a loss of efficacy in 15 patients treated with 7-hexanoyltaxol (QP2)–eluting polymer stents (QuaDS) for in-stent restenosis.4 Although a marked reduction in neointimal growth was achieved at 6 months, angiographic follow-up at 12 months showed a 61.5% rate of restenosis.5 The present study presents the histological findings of coronary atherectomy specimens from a subset of these patients.

Methods

Between October and December 2001, 5 patients treated with QuaDS-QP2 stents (Boston Scientific Corporation) underwent directional coronary atherectomy (DCA) at 11.2±1.0 months for recurrent in-stent restenosis. The patients were from a recently published study of 15 consecutive patients with elective indication to receive QuaDS-QP2 stents in a registry of compassionate use for treating in-stent restenosis from previous bare metal stents.4 All patients were prescribed aspirin (≥100 mg daily) and ticlopidine (500 mg daily) indefinitely, and an elective 6-month angiographic follow-up was scheduled. Four patients received a single 13-mm-long QuaDS-QP2 stent, and in 1 patient, two 17-mm-long stents were positioned, leaving an unintentional gap between them. After the occurrence of late in-stent restenosis in 2 patients with patent stents at 6 months, a decision was made to perform repeat coronary angiography at 12 months.

Paclitaxel Derivate–Eluting Polymer Stent System

The QuaDS drug-eluting stent is a slotted 316-L stainless steel tube covered by multiple nonbiodegradable polyacrylate sleeves that release the more hydrophobic derivative of paclitaxel, 7-hexanoyltaxol (called QP2 or “taxen”). Approximately 800 μg of the drug are loaded per 2.4 mm of sleeve length. The numbers of sleeves vary accordingly to the length of the stent, such that 13-mm-long stents have 3 sleeves (total drug dose=2400 μg), whereas 17-mm-long stents have 4 sleeves (total drug dose=3200 μg).
of smooth muscle cells (α-smooth muscle actin), macrophages (CD68), T-lymphocytes (CD45RO), fibrin II β chain, and the cell cycle antigen (Ki-67).

## Pathological Analysis of Atherectomy Specimens

The samples consisted of multiple fragments of restenotic tissue, although old atherosclerotic plaque was present in 3 of the 4 specimens; no media or adventitia was observed. Morphometric analysis of atherectomy tissue from each patient is summarized in the Table. The mean area of restenotic tissue and old plaque was 4.51±2.07 mm² and 0.22±0.21 mm², respectively. Restenotic tissue was composed of proteoglycan-rich matrix and collagen interspersed with smooth muscle cells (Figure 2, A and B). Fibrin was present focally at the organizing edges of the intima and media (Figure 2C). Picrosirius red revealed a mixture of type III and I collagen representing restenotic tissue and old atherosclerotic plaque, respectively. Two specimens contained numerous CD68-positive macrophages and T-lymphocytes adjacent to areas rich in fibrin (Figure 2, E and F). In proteoglycan-rich restenotic areas, the proliferation index was <1%. In the two specimens with large areas of chronic inflammation, the proliferation rate was as high as 5%. The one specimen examined by transmission electron microscopy showed smooth muscle cells in a collagen-rich matrix with focal areas of fibrin deposition; no inflammation was identified.

### Discussion

The unique hydrophobic properties of paclitaxel cause it to preferentially penetrate into tissues over time so that the arterial wall concentration of the drug exceeds the bulk concentration. Despite the fact that QuaDS stents are loaded with relatively high doses of QP2 (7-hexanoyltaxol), the compound has a lower solubility than the related paclitaxel. Although the drug-release kinetics in humans are unknown, in vivo pharmacokinetic studies in the rabbit iliac arteries demonstrate that ~80% of QP2 is released by 90 days, and the process continues up to 180 days.

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**Figure 1.** Angiographic results of QuaDS-QP2 stents. A, Baseline occlusive in-stent restenosis of the mid left anterior descending coronary artery. B, The lesion was successfully treated with implantation of two 17-mm-long QuaDS-QP2 stents leaving an unintentional space between the two stents (arrow). C, Angiography at 6 months showing patency of the two stents with restenosis in the unprotected space; this segment was treated with re-PTCA. D, At 12 months the vessel appears occluded.

**Coronary Atherectomy Procedure**

Coronary atherectomy was performed with a Flexi-cut DCA system (Guidant) with an 8F guiding catheter. The specimens were immersion-fixed in 10% neutral buffered formalin and processed for paraffin embedding (n=4). One smaller sample was submitted for transmission electron microscopy. Tissue sections from 3 different levels in the block were cut at 4 μm and stained with hematoxylin and eosin, Movat pentachrome, and Alcian blue. Collagen was identified by picrosirius red staining and polarization microscopy. Immunohistochemical staining was performed for the identification of smooth muscle cells (α-smooth muscle actin), macrophages (CD68), T-lymphocytes (CD45RO), fibrin II β chain, and the cell cycle antigen (Ki-67).

**Results**

The patients were 59±11 years old (range, 49 to 66 years). Of the 5 lesions, 2 were in the proximal left anterior descending artery, and the remaining 3 in the left main, intermediate branch, and right coronary artery. In all cases, 6-month quantitative angiography showed QuaDS-QP2 stent patency with an in-stent late loss of 0.19±0.61 mm. One of the patients had target lesion revascularization at 6 months because of focal restenosis in a gap between two patent QuaDS-QP2 stents (Figure 1). Another patient, without restenosis, had target lesion revascularization in the unprotected space; this segment was treated with re-PTCA. D, At 12 months the vessel appears occluded.
In a small study of QuaDS-QP2 stents for the treatment of de novo lesions, the binary restenosis rate was 0% compared with 54% in the control group (bare metal stents [Q-M]) at 18-month follow-up. Intraplascular ultrasound results in 14 patients with QuaDS-QP2 stent implants showed little increase in the initial MLD at 8.3±2.1 months. Although the early safety studies were promising, the larger SCORE (Study to Compare Restenosis rate between QueST and QuaDS-QP2) trial was terminated because of a high 10.2% major adverse cardiac event rate at 30 days in the QuaDS-QP2 group, which was attributed to late stent thrombosis. In the first clinical registry of 15 consecutive patients implanted with QuaDS-QP2 stents for in-stent restenosis, 6- and 12-month angiographic restenosis rates were 13.3% and 61.5%, respectively. It was speculated that the delayed restenosis at 12 months might be related to toxic tissue levels of the drug (≥2400 μg) and/or an inflammatory reaction to the polymer sleeve.

The histological findings of coronary atherectomy specimens from late restenosis lesions in QuaDS-QP2 stents are remarkably similar to the experience with paclitaxel-eluting stents in animals. Persistent fibrin accumulation was found along with smooth muscle cells and proteoglycan- and collagen type III–rich matrix with or without chronic inflammation. The neointimal changes in 28-day animal studies consist of fibrin deposition around stent struts, chronic inflammation, minimal smooth muscle cells, proteoglycan matrix, and incomplete endothelialization.

Studies in rabbit iliac arteries suggest that the reduction in neointimal growth with paclitaxel is dependent on the dose and its release kinetics from the stent. Rapid-release drug-eluting stents with a biodegradable chondroitin sulfate polymer loaded with 42.0 and 22.2 μg paclitaxel showed a dose-dependent decrease in neointima formation at 1 month with evidence of delayed healing; however, this benefit was lost by 3 months. Although stents coated with poly(lactide-co-ε-caprolactone)-co-polymer loaded with 200 μg paclitaxel show persistent neointimal inhibition for up to 6 months, the neointimal is still incompletely healed. In humans, the healing-repair response to bare stainless steel stents is delayed as compared with animals. Human autopsy studies of stainless steel coronary stents suggest that it takes 3 to 6 months for complete healing, whereas in animals, it takes only 28 days. In QuaDS-QP2 stents, it is also possible that an inflammatory reaction to the polymer sleeve was the primary cause of delayed healing and not the drug, although as with most reactions to polymers, giant cells were not observed.

The pathological results of atherectomy specimens from QuaDS-QP2 stents cannot be directly applied to other drug-eluting stents, particularly those coated with paclitaxel or derivatives thereof. Potential problems such as the nonerodible thick polymer sleeve, very high concentration of the active drug, extended release kinetics, loose stent architecture, and inhomogeneous drug delivery (possibly affected by the interspace polymer sleeve) may have compromised the performance of the QuaDS-QP2 stent. Thus, the overall clinical success of any drug-eluting stents may be dependent on multiple design factors and not the drug alone.

References
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